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# Assessment of the Variability of Biogas Production from Sugar Beet Silage as Affected by Movement and Loss of the Produced Alcohols and Organic Acids

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**Abstract:** The biochemical methane potential and composition of sugar beet pulp silage were investigated using samples taken from six different depths in both open and closed silos (height 3.6 m). The biochemical methane potential (BMP) of pulp silage in open silos ranged from 337 to 420 normal litre (NL) $CH_4$/kg volatile solids (VS), while the BMP of pulp silage in closed silos varied between 411 and 451 NL $CH_4$/kg VS. The biochemical methane potential peaked at a depth of 1.45 m with 420 NL $CH_4$/kg VS for open silos and 451 NL $CH_4$/kg VS for closed silos. The ethanol concentration and biochemical methane potential showed the same trend with depth throughout the silos. The energy loss correlated to the loss of volatile solids, and the depths described a linear relationship between them for both the open and closed silos ($R^2$ = 0.997 for the open silo and $R^2$ = 0.991 for the closed silo). The energy potentials and composition of beet pulp silage were highly stratified and there was a risk that the silage samples were not representative in investigations of biomass quality for energy production.

**Keywords:** anaerobic digestion; *Beta vulgaris*; ensiling; renewable energy; organic components; biomethanation

## 1. Introduction

Biogas needs to become a major contributor to green energy production if the EU is to meet its target of 55% of gross final energy consumption being provided by renewable energy by 2050 [1,2] and if the Danish government is to meet its goal of 100% of energy consumption being provided by renewable energy [3]. Wind power will probably provide 60%–80% of total electricity consumption, including transport uses such as plug-in vehicles, in a Danish non-fossil energy scenario [4]. As the contribution from wind power will fluctuate over the year, the remaining 20%–40% must come from other reliable renewable energy sources, primarily biomass [4,5].

The Danish government has set a 40% target for the use of animal manure in biogas production by 2020, whereas in 2012 just 7%–8% of animal manure was used for biogas production [6]. This means that animal manure use for anaerobic digestion (AD) has to increase about tenfold [7,8]. The energy content of animal manure is low due to its high water content, and its organic matter is not easily digestible, therefore co-digestion with easily digestible organic biomass is required if biogas production is to be profitable [9,10].

Agriculture can potentially contribute up to 10 Mt extra biomass that can be used for bioenergy production [11]. Studies show that sugar beet is a good co-substrate for biogas production [12,13] and provides high yields in northern climates, *i.e.*, 120–140 tonnes of beet and tops per hectare [14]. There is a conflict in that beet is harvested once every spring whereas biogas production must be continuous year round. During storage of wet biomasses large amounts of organic matter may be lost due to its transformation into carbon dioxide and methane, as reflected by a 40%–50% loss of carbon (C) when storing animal manures without a cover [15]. Ensiling is a well-known method for storing livestock feed with a high water content over a long period [16], and it has been shown to have positive effects on methane ($CH_4$) yield owing to an increase in easily degradable components with a high biochemical methane production (BMP) potential during storage [17–19]. During ensiling, water and volatile and energy-rich acids and alcohols are produced [20]. These components may be lost due to volatilisation [21] and displaced by being transported in water to the bottom of the silo from where dissolved organic fractions may be leaked. The hypothesis of this study was that the BMP of silage may vary significantly as affected by poor storage conditions and also due to movement of the organic components produced. Therefore, beet roots were ensiled in closed and open containers and samples taken at different depths to assess variation in the BMP caused by movement and losses of dissolved acids and alcohols.

## 2. Materials and Methods

### *2.1. Ensiling Process*

Whole sugar beets were topped and harvested in November 2012, and the whole roots were stored on the farm from November to February in a clamp covered with straw. The air temperature and the temperature of the beets were low, both around 0 °C. Since the storage temperature was relatively low during this period, it was assumed that the storage of whole beets from November to February may only have had a relatively limited effect on the results of the storage trial.

On 12 February 2013, the roots were chopped finely using a Tim Envipro SD-1600 (Tim Envipro, Tim, Denmark). On 13 February 2014, the finely chopped roots (beet pulp) were transported and loaded into six silos outdoors. The silos were 3.3 m high with a diameter of 1.0 m (corresponding to a volume of approximately 3 $m^3$). Three of the silos were left open, *i.e.*, exposed to air, rainfall *etc.* The other three silos were covered with an airtight lid. During the first three weeks, however, a fan was mounted on each lid of the three closed silos to create an exchange of air in the headspace with ambient air through a 4 mm tube. This was done to simulate the air exchange at the top of an open silo and in order to measure emissions from the silage during the first phase (data not presented). After three weeks, the headspace air pressure of the closed silos was maintained at ambient air level through a rubber tube several metres long with a diameter of approximately 4 mm connected to an opening in the lid covering the silos. The air exchange in the closed silos was assumed to be very limited during the remainder of the storage trial.

### *2.2. Sample Collection*

On 4 September 2013, the storage experiment ended and a series of samples was taken from each silo. However one of the open silos was leaking and was therefore excluded from the experiment. Some settling had occurred during the ensiling process, resulting in the surface of the pulp being 0.7–1.15 m below the upper edge of the silo. The sampling depth was measured in each individual silo as the depth from the surface of the pulp after settling during storage.

The upper 0.2 m layer of the pulp was removed from each silo by a dredge pump, and samples were taken at this depth by extracting approximately one litre of pulp from four to five positions. These subsamples were mixed and a sample of approximately 1 litre was taken to represent depth no. 1. After this, the next 0.5 m layer was removed by dredge pump and subsamples were taken and mixed to represent depth no. 2. In this way, a total of six samples were taken from each silo at intervals of 0.5 m, with the fifth sampling depth being 0.3 m from the bottom of the silo, and the sixth sampling depth being at the bottom of the silo. The samples from each depth of the open silos were mixed together, as were the samples from the closed silos, to obtain final samples for physicochemical analysis and BMP testing.

### 2.3. Physicochemical Analysis

Sugar beet pulp samples from open and closed silos were stored in a freezer at −18 °C until they were used in physicochemical analyses and BMP tests. Before the analyses were conducted in triplicate, the samples were transferred to a refrigerator to thaw at 8 °C and then transferred to room temperature, where the temperature of the samples rose to 20 °C.

Dry matter (DM) was determined by drying samples at 105 °C according to the standard method [22]. Total Kjeldahl nitrogen (TKN) and total ammoniacal nitrogen (TAN) were determined according to standard procedures [22]. Crude protein was measured by multiplying the difference between TKN and TAN by 6.25 [7]. Volatile solids (VS) and the ash content of the dry matter were determined after placing the dried samples in a muffle furnace at 550 °C for two hours.

During DM measurement, easily volatile compounds, *i.e.*, alcohols and volatile fatty acids, evaporated and it was necessary to measure their concentration in the untreated sample and add them to the oven-dried samples to obtain the corrected DM. Measured dry matter was corrected according to Weissbach *et al.* [23]. Ethanol was measured using high-performance liquid chromatography (HPLC, Agilent 1100, Agilent Technologies Deutschland GmbH & Co. KG, Waldbronn, Germany). Volatile fatty acid (VFA) concentrations from $C_2$–$C_5$ were measured using a gas chromatograph (Hewlett Packard 6890, Ontario, ON, Canada) with a flame ionisation detector and 30 m × 0.25 mm × 0.25 µm column (HP-INNOWax, Agilent Technologies, Santa Clara, CA, USA). Lactic acid was measured by ion exchange chromatography (IC). For alcohol, lactic acid and VFA analysis by chromatography, the samples were diluted with deionised water and filtered through a 0.2 µm nylon membrane filter prior to injection into the GC and HPLC. In the case of VFA determination, the pH value was adjusted to about 2 using phosphoric acid. For the VFA calibration curve, nine standard solutions containing six VFAs were used in triplicate, with concentrations ranging from 0.25 to 100 mM.

### 2.4. Determination of Biochemical Methane Potentials

The German standard method [24] was used to determine the BMP. Inoculum was collected from an industrial biogas plant on Funen Island, which processes 75% animal manure as prime feedstock and 25% industrial food processing waste by mass at a mesophilic temperature (41 °C). The inoculum obtained was degassed in an incubator for two weeks at 37 °C. DM, VS and pH were 47.8 (±2.5) g/kg, 29.6 (±1.2) g/kg (62% (±3%) DM) and 7.45 (±0.03) respectively.

The inoculum-to-substrate ratio (I/S ratio) was set at 3 based on DM [7,25–29] and all the experiments were performed in triplicate. In addition to measuring gas production from the biomass, the measurement included inoculum for correction of gas production as a blank test, using microcrystalline cellulose (Avicel® PH-101, Sigma-Aldrich, St. Louis, MO, USA) as the reference material. The BMP of the microcrystalline cellulose was 371.1 (±5.0) NL $CH_4$/kg VS and the ratio of BMP to theoretical BMP (TBMP) was 89.4%. The TBMP of cellulose was 415 NL $CH_4$/kg VS.

After mixing the substrate and degassed inoculum, 150 mL nitrogen-flushed buffer medium was added to the inoculum substrate mixture according to the ISO standard method [30,31]. In order to provide a fully anaerobic system, the batch digesters were flushed with nitrogen gas.

The gas chromatograph (7890A, Agilent Technologies, Santa Clara, CA, USA), equipped with a thermal conductivity detector and a 30 m × 0.320 mm column (J&W 113-4332, Agilent Technologies, Santa Clara, CA, USA), was utilised for gas concentration analysis.

Anaerobic digestion was run under mesophilic conditions at 37 °C for about 60 days. Digesters were shaken manually to avoid layering and encourage degassing. A syringe (Hamilton Super Syringe) was used to measure wet biogas volume, and afterwards gas volumes were corrected to dry biogas as normal litres. The biomethane concentration in dried biogas was determined from the gas chromatography analysis, and the determined biomethane corrected to standard condition (273 K and 101.325 kPa) according to [24] and based on the equation below:

$$V_0^{dry} = \frac{V\left[(P - P_w) \times T_0\right]}{P_0 \times T} \quad (1)$$

where $V_0{}^{dry}$ is the volume of the dry biogas or methane in the standard temperature and pressure (NL); $V$ is the measured volume of the biogas or methane (L); $P$ is the pressure of the gas at the time of reading (kPa); $P_w$ is the vapour pressure of the water which is a function of the temperature of the ambient space (kPa); $T_0$ and $P_0$ are normal temperature (273 K) and normal pressure (101.3 kPa) respectively; and T is the temperature of the fermentation gas or of the ambient space (K).

## 3. Data Analysis and Calculations

### *3.1. Theoretical Biochemical Methane Potential*

The measured total acid number (TAN) for all samples was close to zero. The crude lipids content was assumed to be 0.5% of dry matter, as reported in [32]. The carbohydrates were assessed by subtracting all the measured VS components from total VS (Equation (2)) [8].

VS was considered to be composed of VFAs, lactic acid, ethanol, crude proteins, crude lipids and carbohydrates. The molecular formulae of lactic acid and ethanol are $C_3H_6O_3$ and $C_2H_6O$, respectively, and the empirical formulae of VFAs, crude proteins, crude lipid and carbohydrates are $C_2H_4O_2$, $C_5H_7O_2N$, $C_{57}H_{104}O_6$ and $C_6H_{10}O_5$, respectively [33]. Using these empirical formulae, the mass balance equation for volatile solids is written as follows:

$$\text{Total } VS = VS_{VFAs} + VS_{lactic\ acid} + VS_{ethanol} + VS_{crude\ proteins} + VS_{crude\ lipids} + VS_{carbohydrates} \quad (2)$$

Bushwell's formula was used to calculate the theoretical biochemical methane potential (TBMP) of a specific compound with defined molecular formulae under standard conditions (273 K and 101.325 kPa) [34,35]:

$$C_nH_aO_bN_C + \left(n - \tfrac{a}{4} - \tfrac{b}{2} + \tfrac{3c}{4}\right) H_2O \rightarrow \left(\tfrac{n}{2} + \tfrac{a}{8} - \tfrac{b}{4} - \tfrac{3c}{8}\right) CH_4 + \left(\tfrac{n}{2} - \tfrac{a}{8} + \tfrac{b}{4} + \tfrac{3c}{8}\right) CO_2 + cNH_3 \quad (3)$$

$$B_{th} = \frac{(n/2 + a/8 - b/4 - 3c/8)\,22400}{12n + a + 16b + 14c}\ \text{NL CH}_4\ \text{kg}^{-1}\ VS \quad (4)$$

According to Equation 3, the TBMP of VFAs, lactic acid, ethanol, crude proteins, crude lipids and carbohydrates were 373, 373, 730, 496, 1014 and 415 NL $CH_4$/kg VS respectively. Using these values, the theoretical biochemical methane potential could then be calculated as follows:

$$\begin{aligned} TBMP = (373 \times VS_{VFAs} + 373 \times VS_{lactic\ acid} + 730 \times VS_{ethanol} + 496 \times \\ VS_{Crude\ proteins} + 1014 \times VS_{crude\ lipids} + 415 \times VS_{carbohydrates}) \end{aligned} \quad (5)$$

where the *TBMP* is given as NL $CH_4$/kg VS and $VS_{\text{component}}$ is given as the content of the component in total VS (g/g VS).

Data were assessed using analysis of variance (ANOVA) and Tukey's method was used for a multi-comparison test with a significant level of $\alpha = 0.05$ in all cases using SAS 9.2. By means of this statistical analysis, all pairwise differences between factor level means were evaluated. The paired *t*-test was applied to evaluate the difference between data from open silos and closed silos. The average amount of each measured value was calculated and is reported in this paper with the standard deviation written in parentheses.

## 4. Results and Discussion

### *4.1. Characteristics of Fresh Sugar Beet Pulp and Silage Samples*

The DM, VS and ash content of fresh sugar beet pulp were measured as 194.6 (±5.9), 153.4 (±1.8) g/kg and 41.2 (±3.4) g/kg respectively. In the fresh sugar beet, the alcohol and lactic acid concentrations were zero and the VFA concentration was negligible. In samples from the closed silos and open silos, dry matter ranged from 159.3 (±7.8) to 208.7 (±4.3) g/kg and 110.5 (±4.8) to 192.9 (±3.8) g/kg respectively, showing lower overall amounts than DM of fresh sugar beet pulp.

The DM, VS and ash content are depicted in Figure 1. The difference in ash content was fairly large at different depths, being approximately twice as low in the middle than at the surface and at the bottom. Due to the higher density of soil and sand than that of pulp and water, soil and sand sedimented to the bottom of the silos, and this presumably explained the higher ash content in the lower parts of the silos. In samples taken from the top, VS was transformed into highly volatile compounds, some of which evaporated into the atmosphere, causing the higher ash content. Due to the inhomogeneous distribution of water and soil throughout the silos, most of the ash at the top and bottom of the silos could be soluble and insoluble ash, respectively. Owing to the inhomogeneous distribution of water and soil, most of the solid part in the middle of the silos was sugar beet pulp that can absorb more water than sand, leading to a trough in the dry matter profile throughout the silos. Ensiling of biomass with a high moisture content may cause problems with bacteria of the clostridia genus. The moisture content of sugar beet pulp before ensiling was 805.4 (±6.9) g/kg, which was fairly high, and the silage was vulnerable to spoilage, resulting in organic matter and energy losses.

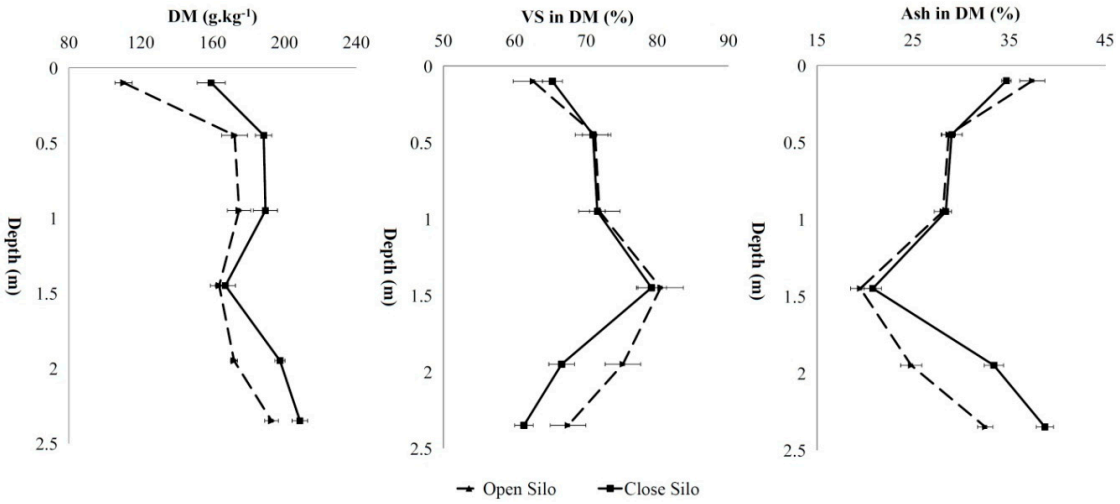


**Figure 1.** DM, ash and VS content in dry matter in ensiled sugar beet pulp by depth from the pulp surface. Bars indicate standard deviation from laboratory replicates.

The average crude protein concentration was 9.43 (±1.44) g/kg and 9.00 (±0.58) g/kg of wet weight (ww) for open silos and closed silos respectively, with the content not differing significantly ($p > 0.05$). In both silos, the main VFA produced was acetic acid, while the concentrations of isobutyric acid and isovaleric acid in both silos were respectively very low and negligible. Due to the availability of oxygen at the top of silos, heterofermentative lactic acid bacteria were able to convert lactic acid to acetic acid, leading to the higher concentration of acetic acid compared to the lower layers. In both silos, the butyric acid concentration was highest in the top layer (1.49 g/kg ww for both open and closed silos), while in the layers below 0.1 m the concentration was low (0.05 to 0.24 g/kg ww). This could be because a lack of moisture inhibits *Clostridia* [20] and the moisture content in the top layer may enhance the activity of *Clostridia*, converting lactic acid into butyric acid, hydrogen and carbon dioxide [36,37], which was also reflected in the fact that lactic acid concentration was lower in the top layer. The pH results were below 4 in both silos, ranging from 3.5 to 3.7, demonstrating no great difference between pH at different depths in either type of silo. Due to the low pH of the substrates, it was adjusted by buffer medium to 7 in incubation bottles in order to prevent any inhibition during anaerobic digestion. Lactic acid and VFAs (mainly acetic acid) showed opposing trends at all silo depths, expressing the conversion of lactic acid to acetic acid by *Clostridia* and heterofermentative lactic acid bacteria.

### *4.2. Methane Yield and Organic Composition of Silage Samples*

The physicochemical characteristic of the silage samples and fresh sugar beet pulp are shown in Table 1. Among the samples from the open silos, the crude protein, VFA and lactic acid content in VS of the sample taken from 0.1 m were highest, while the carbohydrate and ethanol content were lowest compared to the layers further down. The same was found in the closed silos, however the difference in the content of the mentioned components in VS between the surface and the layers lower down was not particularly significant. Transformation of organic matter during storage changed the chemical composition and this change had an impact on methane yield produced by anaerobic digestion.

The BMP of the samples from the open silos ranged from 337 (±3) to 420 (±7) NL CH4/kg VS, while in samples taken from the closed silos it varied between 411 (±5) and 451 (±4) NL $CH_4$/kg VS. The BMP of fresh sugar beet has been reported to be between 361 and 374 NL $CH_4$/kg VS [32]. The BMP in this study was related to the content of volatile solids, which was corrected by including ethanol and VFAs. This correction led to a 15%–20% reduction of measured BMP per kg VS. These results highlighted the importance of correct determination of DM to avoid overestimation of the BMP.

The BMP results, VFAs, lactic acid and ethanol content in VS throughout the silos showed that there was practically the same trend in ethanol and the BMP of samples from both open and closed silos. For instance, in the top layer of the open silos, the ethanol content and BMP were lowest. $CH_4$ and biogas production from samples taken from the middle part of the silos was highest.

The BMP of samples from closed silos (mean BMP = 428 (±12) NL $CH_4$/kg VS) were higher ($p < 0.05$) than for samples from open silos (mean BMP = 389 (±10) NL $CH_4$/kg VS), reaching peak values at the 1.45 m depth in both silos, with 420 (±7) NL $CH_4$/kg VS for open silos and 451 (±4) NL $CH_4$/kg VS for closed silos.

A small amount of VS is used for bacterial growth and metabolisms, not just for methane formation. Previous studies have reported a fairly wide range of bacterial growth: 3% [14], 7% [38] and 5%–15% [35]. Nevertheless, biodegradability assessed using the ratio of BMP to TBMP does not take the transmission of VS to bacterial growth into account, assuming that microbial cellular yield is negligible. In the case of both open and closed silos, biodegradability throughout the silos was above 78%. In the present study the anaerobic biodegradability assessed by BMP/TBMP was 86% by mean value, showing that the very high digestibility of the sugar beet pulp silage and the results were in accordance with previous studies.

The difference in the BMP of samples from the open and closed silos was significantly different ($p < 0.05$) according to the paired *t*-test. The BMP of both silos and the difference between the BMP at each depth for each silo were evaluated according to analysis of variance (ANOVA) and Tukey's method. The results showed that, statistically, the BMP of open silos could be divided into three groups (a, b and c) and there was a significant difference between the results at the surface and at the other depths ($p < 0.05$). In the case of the closed silos, the BMP was statistically separated into two groups (a and b) and, compared with the open silos, the variation in the BMP was moderate.

### *4.3. Mass and Energy Loss after Ensiling*

Ensiling apparently increases methane yield based on organic matter. However it is important to assess methane yield according to wet mass. Table 2 shows methane yield based on wet mass, BMP based on original VS, organic matter and energy losses. Methane production based on wet mass in closed silos was greater than in open silos at all depths, and in both silos the highest methane production per wet mass was observed at the 1.45 m depth. In the open silos, the methane production from the depth of 1.45 m was statistically different from the others, while in the closed silos the methane production from the 0.45 m depth to the bottom of silo was statistically the same. Methane production based on the total wet mass was lowest in the first layer of both silos. Due to more or less the same VS content in the other layers in both silos, the trend of methane production per wet mass and BMP was the same throughout the silos.

**Table 1.** Organic composition, methane yield and hydrolysis kinetic constants of experiments for open and closed silos.

| | Depth (m) | DM (g/kg) | VS (g/kg) | Crude Proteins (% VS) | Crude Lipids (% VS) | VFAs (% VS) | Ethanol (% VS) | Lactic Acid (% VS) | Carbohydrates (% VS) | BMP (Nl/kg VS) | TBMP (Nl/kg VS) | $k_{hyd}$ ($day^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Open silo | 0.1 | 110.5 (±4.8) | 69.2 (±5.7) | 16.0 (±1.5) | 0.8 (±0.00) | 17.7 (±2.8) | 6.9 (±1.0) | 32.1 (±3.8) | 26.5 (±1.6) | 337 (±3) [a] | 434 | 0.189 |
| | 0.45 | 172.3 (±7.2) | 122.9 (±9.3) | 7.8 (±1.1) | 0.7 (±0.05) | 6.6 (±0.9) | 14.7 (±2.8) | 25.7 (±2.3) | 44.5 (±3.4) | 383 (±2) [b] | 458 | 0.180 |
| | 0.95 | 174.8 (±6.5) | 125.7 (±4.6) | 8.0 (±0.7) | 0.7 (±0.04) | 5.5 (±0.6) | 15.4 (±3.2) | 25.1 (±1.7) | 45.3 (±2.8) | 402 (±12) [b,c] | 461 | 0.173 |
| | 1.45 | 163.7 (±4.9) | 131.7 (±2.7) | 7.9 (±1.2) | 0.6 (±0.01) | 5.0 (±0.2) | 15.0 (±2.8) | 24.2 (±1.4) | 47.3 (±3.6) | 420 (±7) [c] | 460 | 0.162 |
| | 1.95 | 172.0 (±1.9) | 129.3 (±6.8) | 5.9 (±0.3) | 0.6 (±0.05) | 5.1 (±0.7) | 14.4 (±2.4) | 25.4 (±0.9) | 48.6 (±1.8) | 403 (±8) [b,c] | 456 | 0.176 |
| | 2.35 | 192.9 (±3.8) | 130.2 (±9.7) | 6.0 (±0.4) | 0.8 (±0.1) | 4.9 (±0.1) | 13.0 (±1.6) | 24.8 (±2.3) | 50.5 (±4.1) | 388 (±8) [b] | 453 | 0.186 |
| Closed silo | 0.1 | 159.3 (±7.8) | 104.1 (±2.4) | 8.2 (±0.4) | 0.7 (±0.06) | 10.1 (±0.5) | 10.6 (±0.3) | 26.8 (±0.4) | 43.6 (±2.1) | 413 (±20) [a] | 444 | 0.167 |
| | 0.45 | 188.5 (±4.6) | 133.8 (±1.5) | 7.4 (±0.6) | 0.8 (±0.00) | 5.2 (±0.1) | 12.4 (±1.0) | 24.0 (±2.2) | 50.2 (±3.1) | 411 (±5) [a] | 452 | 0.173 |
| | 0.95 | 189.5 (±6.7) | 135.7 (±6.0) | 6.9 (±0.1) | 0.8 (±0.03) | 4.5 (±0.2) | 15.3 (±0.8) | 23.0 (±1.5) | 49.5 (±4.4) | 421 (±9) [a,b] | 461 | 0.164 |
| | 1.45 | 167.2 (±5.6) | 132.5 (±9.9) | 6.3 (±0.3) | 0.6 (±0.05) | 4.2 (±0.0) | 15.2 (±1.0) | 23.2 (±0.7) | 50.5 (±5.3) | 451 (±4) [b] | 460 | 0.145 |
| | 1.95 | 197.6 (±2.8) | 131.7 (±3.6) | 6.9 (±0.4) | 0.8 (±0.00) | 4.6 (±0.7) | 13.2 (±1.2) | 24.2 (±2.6) | 50.3 (±3.4) | 437 (±4) [a,b] | 454 | 0.163 |
| | 2.35 | 208.7 (±4.3) | 128.0 (±4.2) | 6.8 (±0.7) | 0.9 (±0.07) | 4.7 (±0.4) | 11.1 (±0.6) | 25.4 (±2.1) | 51.1 (±2.6) | 436 (±17) [a,b] | 447 | 0.169 |
| Sugar beet pulp before silage | - | 194.6 (±5.9) | 153.4 (±1.8) | 5.9 (±0.3) | 0.6 (±0.04) | 0.5 (±0.2) | 0.0 (±0.0) | 0.0 (±0.0) | 93.0 (±1.1) | 342 (±5) | 422 | - |

a, b and c: different superscripts in the same column mean that the BMP for each depth are significantly different.

**Table 2.** VS and energy losses in open and closed silos and methane yield based on original VS.

| | Depth (m) | VS in DM (%) | Ash in DM (%) | BMP (Nl/kg VS) | Methane Yield (Nl/kg Sample) | VS Loss (%) | BMP (Based on Original VS) (Nl/kg VS) | Energy Loss (%) |
|---|---|---|---|---|---|---|---|---|
| Open silo | 0.1 | 62.6 (±2.8) | 37.4 (±1.3) | 337 (±3) [a] | 23.3 (±0.5) | 55.0 (±1.5) | 152 (±1) | 55.7 (±1.1) |
| | 0.45 | 71.3 (±1.8) | 28.7 (±0.7) | 383 (±2) [b] | 47.1 (±0.4) | 33.2 (±1.0) | 256 (±2) | 25.2 (±0.6) |
| | 0.95 | 71.9 (±2.9) | 28.1 (±0.9) | 402 (±12) [b,c] | 50.5 (±1.1) | 31.2 (±0.5) | 276 (±2) | 19.2 (±0.4) |
| | 1.45 | 80.5 (±3.2) | 19.5 (±1.0) | 420 (±7) [c] | 55.3 (±0.6) | −10.5 (±0.6) | 464 (±4) | −35.7 (±0.8) |
| | 1.95 | 75.2 (±2.5) | 24.8 (±1.1) | 403 (±8) [b,c] | 52.1 (±0.7) | 18.7 (±0.7) | 328 (±4) | 4.2 (±0.4) |
| | 2.35 | 67.5 (±2.5) | 32.5 (±0.8) | 388 (±8) [b] | 50.5 (±0.7) | 44.2 (±0.9) | 216 (±3) | 36.7 (±0.7) |
| Closed silo | 0.1 | 65.3 (±1.4) | 34.7 (±0.5) | 413 (±20) [a] | 43.0 (±1.2) | 49.3 (±1.1) | 209 (±6) | 38.8 (±0.5) |
| | 0.45 | 71.0 (±2.5) | 29.0 (±1.1) | 411 (±5) [a] | 55.0 (±0.4) | 34.3 (±0.7) | 270 (±1) | 21.0 (±0.1) |
| | 0.95 | 71.6 (±1.1) | 28.4 (±0.6) | 421 (±9) [a,b] | 57.1 (±0.8) | 32.3 (±0.9) | 285 (±3) | 16.6 (±0.7) |
| | 1.45 | 79.2 (±2.1) | 20.8 (±0.9) | 451 (±4) [b] | 59.8 (±0.3) | −2.6 (±0.3) | 463 (±2) | −35.2 (±0.5) |
| | 1.95 | 66.6 (±1.8) | 33.4 (±1.0) | 437 (±4) [a,b] | 57.6 (±0.2) | 46.3 (±0.9) | 235 (±2) | 31.4 (±0.4) |
| | 2.35 | 61.3 (±1.3) | 38.7 (±0.9) | 436 (±17) [a,b] | 55.8 (±1.0) | 57.4 (±1.0) | 186 (±7) | 45.7 (±0.8) |
| Sugar beet pulp before silage | - | 78.8 (±0.8) | 21.2 (±1.0) | 342 (±5) | 52.5 (±0.3) | - | 342 | - |

a, b and c: different superscripts in the same column mean that the BMP for each depth are significantly different.

The BMP of fresh sugar beet pulp of 342 (±5) NL $CH_4$/kg VS when compared to samples from both silos was lower, but in contrast its methane yield of 52.5 (±0.3) NL $CH_4$/kg sample was quite high and comparable with the silage samples. It is imperative for biogas plants that the energy yield per wet biomass should be high, therefore it is important to prevent mass and energy losses during ensiling.

In order to evaluate the efficiency of silage, it is important that methane yield should be calculated based on original VS. Due to VS and ash stratification, the calculated original methane yields differed at each depth. Original methane yields showed that for all samples except from the depth of 1.45 m, methane yields were lower than those of fresh sugar beet pulp at 342 (±5) NL $CH_4$/kg VS. These results showed that ensiling of sugar beet pulp in this study in most cases led to a decrease in methane yield. Energy losses had the same trend as VS losses in both silos, which were highest at the surface and at the bottom of the silos. As with the VS losses, the calculated energy loss of samples from a depth of 1.45 m was negative, expressing an increase in energy content. Generally, energy losses were lower than VS losses at all depths of the open and closed silos. VS losses are depicted *versus* energy losses in Figure 2 with a related linear equation between them. For the open silo, a loss of up to 16% of VS resulted in no energy loss, whereas in the closed silo this figure rose to up to 22% due to the better silage conditions in the closed silo. On the other hand, the slopes of the lines for open and closed silos were 0.7269 and 0.7279 respectively. The slope of the line might be dependent on the type of feed used in silage, so given that sugar beet pulp was used for silage in both silos the line slopes are very similar.

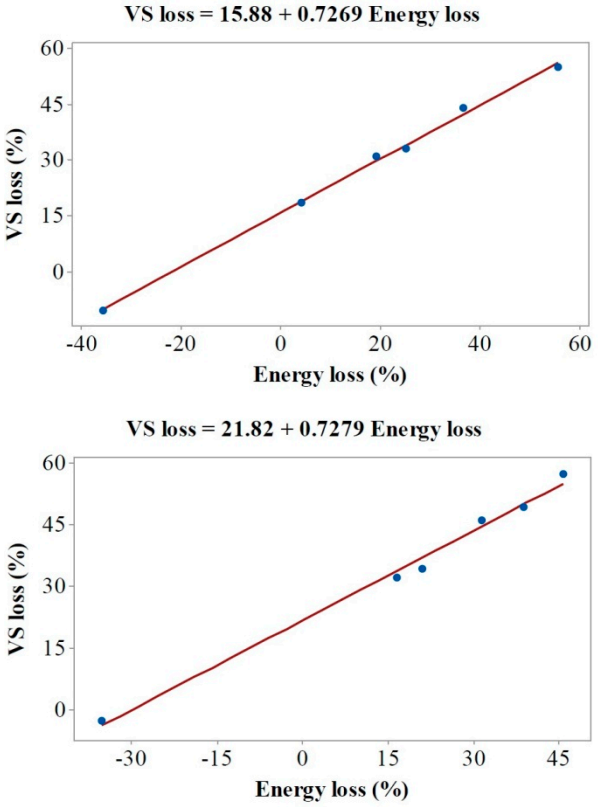


**Figure 2.** Energy losses *versus* VS losses for open silo (**top**) and closed silo (**bottom**).

## 5. Conclusions

This study shows that energy and VS losses vary at different depths due to the movement of material throughout the silos. The energy potentials and characteristics of beet pulp silage were highly stratified between different layers and there was therefore a considerable risk that the samples collected were not representative for a study of the quality of biomass for energy production.

This study found significant energy loss if the ensiling was not performed in an optimum way, highlighting the importance of the control of silage or the utilisation of co-ensiling of low dry matter biomass with a high dry matter one to prevent energy and VS losses. Despite the risk of energy loss, the generation of organic acids and alcohols boosted biogas production with very high biodegradability, showing the significant potential of sugar beet silage in biogas production when ensiling avoids energy loss from surface layers.

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